

Analysis of PCBs and Pesticides

in

Air and Precipitation Samples

**IADN Project
Gas Chromatography Procedure**

prepared by

Ilori Basu

School of Public and Environmental Affairs
Indiana University
Bloomington, Indiana.

I. ROUTINE GC MAINTENANCE

I.A Gas Tanks

1. Check gas tanks. Tanks should not go dry. While changing the tank, lower the temperature of the oven down to 40 C. Leave it at 40 C for about half an hour after changing the tank to get rid of air or oxygen that was drawn in.
2. Check column head pressure. It should be at 24 psi. If pressure falls, tighten the septum nut. If the pressure is still low check for leaks and tighten other connections.

I.B GC Baking

1. After every GC run bake the oven at 280 C for 1 hour. After every other run also bake the injector and the detector at 280 C and 380 C.

I.C. Septum

1. Preparation of Septum
 - a) Place the septa in a small beaker. Cover with aluminum foil.
 - b) Place the beaker in GC oven and bake the septa while baking the column at 280 C.
 - c) Store the clean septa in the beaker keeping the foil lid intact and use as needed. Always use tweezers to get clean septum out.
2. Changing of Septum
 - a) After every 60 samples or so change the septum.
 - b) Cool the oven down to 40 C.
 - c) Remove autosampler towers.
 - d) Remove septum nut and take the old septum out. Discard.
 - e) Using clean Q-tips soaked in hexane, wipe off the septum holder until no more dirt is seen on Q-tips.
 - f) Put in pre-cleaned septum and replace the nut. Nut should be snug but not too tight. Column head pressure should go up to 24psi if nut is tight enough. Check the tightness of the nut after injecting the first sample. Make sure that the head pressure is still 24 psi.

I. ROUTINE GC MAINTENANCE

I.D. Checking Background

1. Background signal in GC varies from 28 to 32. Hexane is analyzed at the start of every GC run to monitor the baseline stability. If the signal goes up or hexane run produces noisy chromatogram GC should be cleaned.

I.E. Checking Standard

1. Mullin 94 standard and Mixed Pesticide Standard should be monitored to check peak detection and peak broadening or tailing. If the peak shapes are not satisfactory, column should be clipped. More than 90 peaks should be detected in PCB standards and cong.17 and 18 should be separated. If not, install a new column.

I.F. Checking Leaks and Gas Flow

1. Check leaks once in two weeks with a leak detector. Check around the septum, at the injector end, and at the detector end of the column.
2. Check the gas flow once in two weeks with a flow meter. Approximate gas flow are as follows :

Split vent	120 ml/min.
Purge vent	2 ml/min.
Total flow through detector	22 ml/min.

II. GC CLEANING: CLIPPING OLD COLUMN OR INSTALLING A NEW COLUMN

II.A Taking Apart

1. Turn oven, injector and detector off.
2. Turn hydrogen and nitrogen off. Wait until everything cools down.
3. Take the autosampler tower off.
4. Undo the small nut covering the septum and the large nut underneath it to expose the injection liner. Take the liner out.
5. Open the oven. Take the columns out (by detaching from injector and detector ends).
6. Unscrew the nuts from both injector and detector ends of columns and plug the column ends with septum. **Open end of column should not be exposed to air.**
7. Place the columns on the workbench.
8. Unscrew the holder nut underneath the injection liner. There is one gold seal and a washer in it. Washer and seal need to be replaced each time it is taken apart. Clean these parts by ultrasonication with Dichloromethane and Hexane.
9. put a beaker inside the oven underneath the injection port and pour some hexane through the injection port. Clean the injection port with Q-tips and rinse again with hexane.

II.B Assembling Injection Port and Liner

1. After ultrasonication air dry each part. Assemble the holder nut. Place the gold washer first and then the seal. The tapered opening of the seal will face downward. (The tapered end will hold the end of the ferrule from the column.) Screw the nut in before placing the injection liner.
2. Insert a new liner.
3. Put a viton O-ring on the liner. Put the big nut on and tighten it. Put a clean septum. Cover the septum with septum nut. Tighten with wrench.

II. GC CLEANING: CLIPPING OLD COLUMN OR INSTALLING A NEW COLUMN

II.C Clipping Column

1. Take the nut off the injector end of the column. Carefully scrape out all the ferrules from the column nuts. Clean all different parts with Q-tips soaked in DCM and ultrasonicate these with DCM and Hexane for 10 minutes with each solvent. Onto the column, insert the nut first and then the ferrule with conical end pointing towards the open end of the column.
2. Clip the column. Make a clean cut with diamond tip score or ceramic square. Examine the hole with magnifying glass. It should be a clean hole without any jagged end. **Always clip the column after putting the nut and the ferrule on.**
3. Measure **25mm** from the tip of the column. Mark this point with Liquid Paper®.
4. Carefully insert the column with nut and ferrule through the holder nut and screw it in. As soon as it feels tight, pull the column out gently until the white mark is seen. Hand tighten the screw more and make it tight with wrench 1/4 turn after hand tight. **Do not overtighten.**
5. Take the nut off the detector end of the column. Put the nut and the ferrule on the column in the same way as in the injector end. Clip the column and check for the nice clean cut. Insert the column all the way up until it does not go any more. Pull down about 1 mm and tighten the screw. Turn hydrogen on and check the flow of gas through the column by inserting the cut end in a beaker of hexane. Turn hydrogen off.

II.D. Checking Leaks and Gas Flow

1. Turn H₂ and N₂ on. Check leaks with a leak detector. Check around the septum, at the injector and at the detector end of the column. Check that the head pressure is 24 psi.
2. Check the gas flow with a bubble meter. Approximate gas flow are as follows :

Split vent	120 ml/min
Purge vent	2 ml/min.
Total flow through detector	22 ml/min

II. GC CLEANING: CLIPPING OLD COLUMN OR INSTALLING A NEW COLUMN

II.E. Assembling

1. Replace autosampler tower.
2. Turn heated zones on.
3. Turn oven on and set the temperature to 40 C for an hour. Change oven temperature to 70 C and leave another hour.
4. If it is an old column, bake the column, injector and detector for an hour.

Baking temperature :

Oven	:	280 C
Injector A	:	280 C
Injector B	:	280 C
Detector A	:	380 C
Detector B	:	380 C

5. If it is a new column, bake injector and detector. Column can be baked by ramping it 1 or 2 degrees per minute to 280 C. Hold there for 1 hour.
6. If blank run looks satisfactory, put in a standard and check.

III. ROUTINE GC OPERATION

III.A. GC condition and oven temperature program:

PCBs, Hexachlorobenzene, and DDE are eluted in the hexane fraction, whereas the other chlorinated pesticides are eluted in the 50% dichloromethane in hexane fraction after silica gel column chromatography. The procedure for nitrogen blowdown, spiking with internal standard, and making microvials for the autosampler are described in IADN Project Sample Preparation Procedure, Version 1.0, June ,1995.

Gas Chromatograph used for analysis of PCBs and pesticides is Hewlett Packard 5890 with 7673A Autosampler. Operation is controlled by the Integrator Hewlett Packard 3396. Data acquisition is done by Hewlett Packard Peak 96. Finally, data

analyses are done in Hewlett Packard 3365 ChemStation.

Carrier gas:	Hydrogen
Make up gas	Nitrogen
Split vent:	120 ml/min
Purge vent	2 ml/min
Total flow through the detector:	22 ml/min
Column:	DB-5, 60m, 0.25mm i.d, 0.1 μ film thickness

Temperature Program:	Initial temp.	100 ⁰ C
	Initial time	1 min.
	Rate	1 ⁰ C/min
	Final temp.	240 ⁰ C
	Rate A	10 ⁰ C
	Final temp A	280 ⁰ C
	Final time	20 min.
	Purge on	3 min.
	Purge off	150 min.
Run time	165 min.	

The GC condition, column type, and the oven temperature program was specified by Mike Mullin. The method name is Mullin.met.

III.B. GC Pre-run

1. Check to see if there is sufficient H₂ for operation. If not, change tanks.
2. If necessary, change septum.
3. Bake oven at 280 C for 1 hour. Bake injector and detector at 280 C and 380 C respectively about every other time the oven is baked.
4. Cool oven to 100⁰C, injector to 250 C, and detector to 350 C.
5. Make the samples ready in microvials and load the autosampler tray.

III. ROUTINE GC OPERATION

III.C. Preparing Sequence in ChemStation

1. Editing Sequence

- a) Sequence Parameters

Sequence Edit Sequence Parameters

Type in operator's name and subdirectory name. Type in information about calibration standard and spikes in comment.

b) Sequence Table

Sequence Edit Sequence Table

Enter "From Vial" and "To Vial" number.

c) Sample Table

Sequence Edit Sample Table

Type in sample ID's. First vial should be hexane. Second vial should be calibration standard. Third vial should be a Performance standard. Other vials are actual samples. At the end of each sample ID indicate whether the sample is a hexane fraction or 50% fraction (H,F1). Repeat hexane blank and a fresh standard after every set of samples.

f) Print Sequence

Sequence Print

x Sequence Parameters
x Sample Table

III. ROUTINE GC OPERATION

III.D. Starting a GC run

1. Programming integrator

Set runnum 1

Set Date mo/day/year

Shift edit seq The integrator will ask questions about method to load and autosampler sequence. Enter all information.

2. Programming peak 96

Integrator 2

Follow the keys:

Data Acquisition Set up integrator Select old method

Mullin.met Download method Mullin.met

3. Set up PC

Follow the keys:

Generate new

Data prefix J3095 (June 30, 95. It will pick up the vial number from the integrator)

Check export path/peak/export 2

Data to ChemStation Y

3. Transfer information from integrator to PC

Follow the keys:

Utilities Transfer Integrator to PC IBCOL.SEQ

4. Start a GC run

follow the keys:

Data Acquisition Start Seq

5. Post GC run

After the GC run is over copy the .d files on floppy disks.

IV. HP 3365 CHEMSTATION INITIALIZATION AND BASELINE CORRECTION

IV.A. Copy the .d files from Peak 96 on to the floppy disks.
Copy the .d files to HP 3365 ChemStation.

IV.B. Load IBPCBN.MTH or IBPEST2.MTH

IV.C. Loading Chromatogram

Files Load Data File

Subdirectory Sample Vial Number

Chromatogram will appear on screen.

IV.D. Baseline Correction

a) By Integration Event

Go to "command line" and type in Clrevents.

Correct starting parameters and then make baseline corrections by using Baseline Now, and Area Sum.

Starting Parameters

Integration Integration Events

Initial Area Reject 10 Initial
Initial Peak Width 0.04 Initial
Shoulder Detection OFF Initial
Initial Threshold -4 Initial
Integrator On 0.0
Negative peak on 0.0

b) Integrate the chromatogram

Change the scale axis for close view of each peak.

c) Baseline Now:

This command is used to get a straight base line.

Integration Possible Events Baseline Now

Click cursor where you want the baseline.

IV. HP 3365 CHEMSTATION INITIALIZATION AND BASELINE CORRECTION

Integration Integrate

If do not like changes go to Integration Events
and delete.

d) Area Sum and Negative Peak:

This command is used to split a peak.

Integration Possible Events Neg. Peak

Turn negetive peak on and off in the area to split the peak

Integration Possible Events Area Sum

Move the cursor and click on the area to split.

Notice: Area Sum On\Off does not work with Neg. Peak On.

V. PESTICIDE DATA REDUCTION IN 50% FRACTION

V.A. Standard

1. Integration and Peak Identification

Call standard chromatogram and correct baseline and integrate

Compounds	GC Retention time min.(approx.)	concentration ng/ml
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-HCH	35	20
-HCH	40	20
Cong 65(ISTD)	59	20
-Chlordane	70	20
cong 155(ISTD)	72	20
-Chlordane	73	20
t-Nonachlor	74	20
Dieldrin	77	20
DDD	86	20
DDT	93	20
Dibutylchloredate	110	10

2. Printing Report and Saving Text File

Graphics Annotate

Type in the sample name and add it on the chromatogram.
Check Calibration Settings under Reports.

Mixed Pest. Standard.

Ref. Window 1
Nonref. Window 1
Units of Amount ng
Sample ISTD 20
Fit: Linear Origin: Force

Check report on screen before printing

Reports Specify Report

X File (X Auto) X Area
X Screen X ISTD (if calib. is replaced).
X Report X Percent (if new calib is made).

If the report looks alright, print the report.

Reports Print Report

To print report on paper go back to Specify Report and add
Printer and Chromatogram to the options selected.

V. PESTICIDE DATA REDUCTION IN 50% FRACTION

Print integration events and calibration settings

File Return to Top

Method Print Integration Events

3. Preparing New Calibration

Set up initial calibration table by identifying the standard peaks in the area percent report.

Reports Prp Calib/Recalib. New Table

Manually type in the amount and name of each analyte with the retention times from the area percent printout.
Highlight "yes" in Reference ISTD peak boxes for Congener 65 .

OK Do you want to delete all the peaks with the

amount of zero? Yes Save to method? Yes

You will get a printout for calibration table.

4. Replacing Previous Calibration

For subsequent standard runs with analyte peaks all correctly identified in ISTD report the calibration table is replaced.

Reports Prep Calib/Recalib.

Recalibrate Replace OK

Save to method? Yes

Will get a printout of calibration table.

If the GC column has been clipped or running conditions have been changed the analyte peaks shift so much that they are not found in the internal standard report and then a new calibration table will have to be created.

V. PESTICIDE DATA REDUCTION IN 50% FRACTION

5. Saving Calibration on Data File

File Command Line

Type in: SAVETBL C:\HPCHEM\1\DATA\SUBDIR\0**R0101.CAL

6. Saving Event on Data File

File Command Line

Type in: SAVEE C:\HPCHEM\1\DATA\SUBDIR.\0**R0101.EVT

V.B. samples, 50% fraction

1. Integration - See section IV.D.
2. Printing Report and Saving Text File.
See previous section V.A.2. with the following exceptions:

NOTE: Sometimes it is necessary to increase the window more than 0.25% to find internal standard. If it goes more than 0.5%, rerun the sample in GC.

3. Saving Events - See section V.A.6.

V.C. copying .Txt files

After one set of data is reduced write comments in the text files.

Program manager Accessories Note pad

Click on C, HPCHEM 1, data, subdirectory and .txt files.

Call .txt file one by one and write down comments about spike, dilution, reinjection etc.

Save .txt files after writing comments.

VI. PCB AND PESTICIDE DATA REDUCTION IN HEXANE FRACTION

VI.A. Mullin 94 Standard

1. Integration and Peak Identification

Load standard chromatogram and correct baseline according to IV - B and C. Integrate and divide the chromatogram in five to six sections. Identify PCBs from Mullin's 94 chromatogram and pesticides (HCB and DDE) from Mixed Pesticide Standard.

2. Printing Report and Saving Text Files

Graphics Annotate

Click in text box and type in sample name. Click on
Add, and then on chromatogram where you want sample name to be placed.

Check Calibration Settings under Reports.

Ref. Window	1.0
Nonref. Window	1.0
Units of Amount	ng
Sample ISTD #1	8
Sample ISTD #2	6

Fit: Linear Origin: Force

Check report on screen before printing

Reports Specify Report

<input checked="" type="checkbox"/> File (<input checked="" type="checkbox"/> Auto)	<input checked="" type="checkbox"/> Area
<input checked="" type="checkbox"/> Screen	<input checked="" type="checkbox"/> ISTD (if calib. is replaced).
<input checked="" type="checkbox"/> Report	<input checked="" type="checkbox"/> Percent (if new calib is made).

Reports Print Report

To print report on paper go back to Specify Report and add Printer and Chromatogram to the options selected.

VI. PCB AND PESTICIDE DATA REDUCTION IN HEXANE FRACTION

Print integration events and calibration settings:

File Return to Top

Method Print Integration Events

Calibration setting

3. Preparing New Calibration

Set up initial calibration table by identifying congener peaks from Mullin's chromatogram in the area percent report.

Reports Prep Calib/Recalib.

New Table OK

Manually type in and enter the amount and name of each analyte with the retention times from the area percent printout.

Highlight "yes" in Reference ISTD peak boxes for Congener 30 and 204.

Ref.ISTD#1 will be used up to cong.110.

Ref.ISTD#2 will be used from cong.82.

OK Do you want to delete all the peaks with the
amount of zero? Yes Save to method? Yes

4. Replacing Previous Calibration

For subsequent standard runs with analyte peaks all correctly identified in ISTD report the calibration table is replaced.

Reports Prep Calib/Recalib.

Recalibrate Replace OK

Save to method? Yes

Will get a printout of calibration table.

VI. PCB AND PESTICIDE DATA REDUCTION IN HEXANE FRACTION

If the GC column has been clipped or running conditions have been changed, the retention times of analyte peaks shift and they are not identified in the ISTD report. A new calibration table will have to be created. This can be done either by following Section VI.A.3 or by inserting the new retention time in old calibration table and creating a temporary calibration table. Get ISTD report of the new standard with temporary calibration table and replace the calibration according to Section VI.A.4.

5. Saving Calibration on Data File

File Command Line

Type in SAVETBL C:\HPCHEM\1\DATA\SUBDIRECTORY\0**R0101.CAL

6. Saving Event

File Command Line

Type in: SAVEE C:\HPCHEM\1\DATA\SUBDIR.\0**R0101.EVT Enter with keyboard.

VI.B. Samples, Hexane Fraction

1. Integration - See previous section IV.D.

2. Printing Report and Saving Text File

See previous section V.A.2. with the following exceptions:
Calibration Settings Ref. and Nonref. Windows should be
changed to 0.25%.

NOTE: Sometimes it is necessary to increase the window more than 0.25% to find internal standard. If it goes more than 0.5% rerun the sample in GC.

3. Saving Events - See previous section V.A.6.

VII. CREATING EXCEL FILE FOR PCB ANALYSIS

1. Call *.txt files
2. Select "fixed width" and click on "next"
3. Click on line and "finish"
4. Delete a1..a5
5. Insert 6 rows
6. Delete column C
7. Change column width b to 10
8. Edit and replace *not found* to 0
9. Copy 0's to C

10. Type at A1

Subdirectory
sample name
vial id
date

11. Delete rows A124 to I131

12. Delete negative peaks amount

13. At 124 type "total pcbs"

14. At C124 put formula $=((\text{sum}(c9..c121))-(c15+c16+c43+c69+c90))$

15. At A126 type % recovery of 14 = $c16/20*100$

16. At A127 type % recovery of 65 = $c43/5*100$

17. At 128 type % recovery of 166 = $c90/5*100$

18. At 130 type ratio of 30:204 = $B18/B103$

19. At A132 type HCB = c15

20. At A133 type DDE = c69

Write comments

Save the as .xls files

VIII. DATA STORAGE AND DATA RETRIEVAL

VIII.A. Copy the complete files (*.d, *.txt, *.evt, *.cal, and *.xls) onto the floppy disks. Leave one copy on hard disks too.

VIII.B. Loading Old Chromatogram

Same as Loading chromatogram in section IV.C.

VIII.C. Loading Old Calibration

Program Manager File Manager

Click on c:\hpchem\1\subdirectory\0**R0101.cal

File Copy

To C:\hpchem\1\methods\appropriate method.cal

Replace Calib Yes

Close File manager and Return to TOP
Click on Method and Load proper method.
Make sure that you got correct calibration table by clicking
on

Report Edit Calibration Table

VIII.D. Loading Old Integration Event

Program Manager File Manager

Click on c:\hpchem\1\data\subdirectory\0**R0101.evt

File Copy

VIII. DATA STORAGE AND DATA RETRIEVAL

To C:\hpchem\1\methods\appropriate method.evt

Replace Event Yes

Close File manager and Return to TOP

Click on Method and Load proper method.

Make sure that you got correct integration event by clicking

on

Integration Integration Event